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## **Invasive mosquito species in Europe and Serbia, 1979 – 2011**

Petric, D ; Zgomba, M ; Ignjatovic, C A ; Marinkovic, D ; Bellini, R ; Schaffner, Francis ; Pajovic, I

**Abstract:** People's increased mobility and international trade play important roles in the dissemination of vectors and the pathogens that they could transmit. Climate change is likely to become another important consideration in the near future. The responses of insects to these changes (in addition to potential for increased vector capacity) could allow for a broadening of their colonized areas and the invasion of new sites. In the last couple of years a number of pathogen introductions into Europe have been recorded. The latest (Ravenna, Italy, 2007) was caused by the tropical Chikungunya virus, which is transmitted by the "Asian tiger mosquito", a species introduced into Europe in 1979 (Albania), and then Italy in 1990. Invasion continued to France in 1999 and until present, Belgium, Montenegro, Greece, Switzerland, Croatia, Spain, Bosnia and Herzegovina, the Netherlands, Slovenia, Germany, Serbia, Bulgaria and Turkey have been invaded. Deciphering the true cause of changes in the distribution of mosquitoes is difficult and complex and depends, to a great extent, on the availability of data obtained by monitoring. In order to assist in vector-borne disease preparedness, distribution of the most important invasive species *St. albopicta* in Europe and particulars of findings in Serbia are conferred.

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**International Symposium:  
Current Trends in Plant Protection**

# **Proceedings**

**Belgrade, 25-28. September 2012**

## INVASIVE MOSQUITO SPECIES IN EUROPE AND SERBIA, 1979 - 2011

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People's increased mobility and international trade play important roles in the dissemination of vectors and the pathogens that they could transmit. Climate change is likely to become another important consideration in the near future. The responses of insects to these changes (in addition to potential for increased vector capacity) could allow for a broadening of their colonized areas and the invasion of new sites. In the last couple of years a number of pathogen introductions into Europe have been recorded. The latest (Ravenna, Italy, 2007) was caused by the tropical Chikungunya virus, which is transmitted by the "Asian tiger mosquito", a species introduced into Europe in 1979 (Albania), and then Italy in 1990. Invasion continued to France in 1999 and until present, Belgium, Montenegro, Greece, Switzerland, Croatia, Spain, Bosnia and Herzegovina, the Netherlands, Slovenia, Germany, Serbia, Bulgaria and Turkey have been invaded. Deciphering the true cause of changes in the distribution of mosquitoes is difficult and complex and depends, to a great extent, on the availability of data obtained by monitoring. In order to assist in vector-borne disease preparedness, distribution of the most important invasive species *St. albopicta* in Europe and particulars of findings in Serbia are conferred.

*Key words:* invasive mosquito species, *St. albopicta*, *Ae. albopictus*, Asian tiger mosquito

### INTRODUCTION

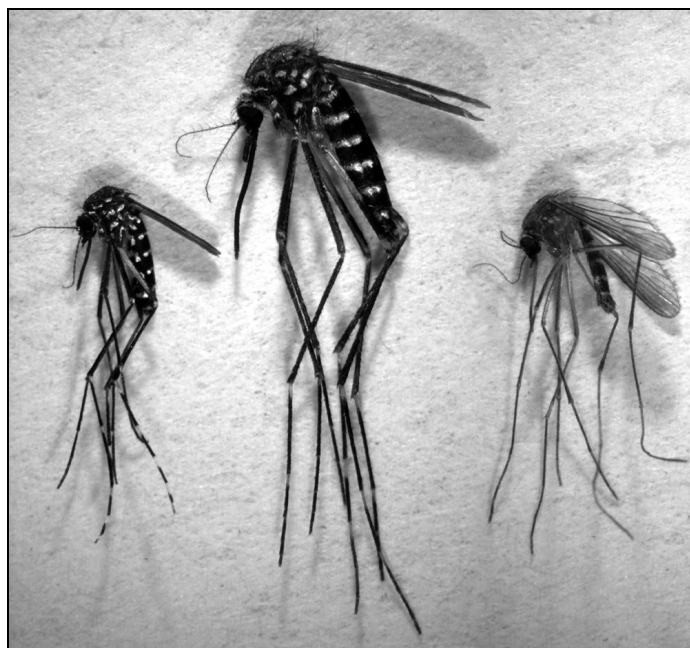
The Asian tiger mosquito is on a rampage. Entomologists are impressed, public health officials are nervous, and many of the rest of us are swatting furiously (Enserink, 2008). Present-day human activities enable the transportation of mosquitoes from one continent to another within a matter of hours to a few days. International trade and the increased transcontinental mobility of humans facilitate the dispersal and, in some cases, the establishment of exotic mosquito species in other countries with favourable climatic conditions. Exotic species are thus shuttled from their native geographic ranges to recipient biotopes where they have never been present before. If some of these exotic species possess mechanisms that allow them to adapt to the new conditions and reproduce in the recipient ecosystem, they are termed "invasive".

Human activities have initiated the spread of invasive mosquito species and vector-borne diseases (a disease that is transmitted to humans or other animals by an insect or other arthropod), and ongoing globalization and increases in air temperature are greatly accelerating the process. As a result, many vector introductions into Europe have been reported since the end of the old millennium. Among the introduced mosquito species, some, such as *Stegomyia albopicta* [*Aedes albopictus*] and *Hulecoeteomyia japonica* [*Aedes japonicus*] are already well established in large areas, whereas others, such as *St. aegypti* [*Aedes aegypti*], *Georgescraigius atropalpus* [*Aedes atropalpus*] and *Hulecoeteomyia koreica* [*Aedes koreicus*] are still confined to their introduction sites or surroundings; others, such as *Ochlerotatus triseriatus* [*Aedes triseriatus*], have thus far only been intercepted during surveillance programs (Schaffner and Van Bortel, 2010)

Three of six mentioned species are notable for their dispersal potential and their significance as vectors of human diseases: *St. aegypti*, *St. albopicta* and *Hl. japonica*. Their desiccation-resistant eggs, wide host preference range, ability to exploit a wide range of natural and artificial breeding places (container-breeding species) and adaptation to temperate climates including winter diapause (except *St. aegypti*) enable the permanent establishment of viable populations in temperate regions (Hawley, 1988; Moore, and Mitchell, 1997). Invasive species pose a threat to biodiversity by homogenizing biota with cosmopolitan species that usually endanger and replace native counterparts. Once misbalanced, the restoration of native diversity becomes impossible. Invasive mosquito species also pose a threat to human and/or animal health as a biting nuisance and as vectors of transmittable mosquito-borne diseases.

All invasive mosquitoes that threaten Europe are container breeding species. For such species, artificial containers with stagnant water such as private and public catch basins, water barrels, cemetery vases, buckets and used tires, are known to be a suitable habitat. For tree hole breeding mosquitoes (that was originally *St. albopicta*) abandoned or outdoor stored tires are nearly ideal place for development. Exposed to the rain, tires can accumulate water, organic material and microbes. In addition they offer high humidity, high temperatures, and lack of natural enemies (Beuwekes et al., 2011). Female mosquitoes can lay their eggs inside these tires, using them as a 'vehicle' to get transported and invade new areas (Reiter, 1988). Eggs of *Stegomyia* and *Hulecoeteomyia* spp. are drought resistant and are oviposited just above the water level. When the water level inside tires rises due to rain (e.g., in a new area where that species did not previously occur) the eggs can hatch and invasion begins.

The results of surveillance of invasive mosquito species that started in Federal Republic of Yugoslavia (FRY), sustained in State Union of Serbia and Montenegro (SCG) and has been continued in both Republic of Montenegro (ME) and Republic of Serbia (RS) will be presented, focusing on *St. albopicta*, the only invasive species at hand (Fig. 1). Biology of other five invasive mosquito species and their distribution in Europe will be discussed.



**Figure 1.** *Stalbopicta* (left) in comparison to invasive *Hl. japonica* (middle) and indigenous *Culex pipiens* (right) – (Schaffner and Hendrickx, 2011)

## MATERIAL AND METHODS

At a beginning of the surveillance in Federal Republic of Yugoslavia (now Montenegro) and Serbia (2001 – 2008) inspections were conducted to determine if exotic mosquitoes were present at a companies that are engaged in the international used tire trade. Later on, surveillance has been continued in factories (e.g. cement factory La Farge, Beočin) that utilize used tires collected within Serbia as a fuel, having in mind that these tires could have been stored close to the borders of Montenegro, FYRO Macedonia (intensive road travel from Greece) and Croatia where *St. albopicta* is widespread. Inspections were done from June through October with varying frequency based on the foreseen risk, ranging from every week for high risk premises to once per season for low risk premises, usually in August and September, when mosquitoes are most abundant.

Tires without water were all, or randomly checked for presence of eggs above the water marks and these with water were visually inspected for mosquito larvae. Mosquito larvae were collected from tires with a pipette and transferred in vials filled with water for consequent breeding or tubes with 70% ethanol. Up to several hundreds of tires were inspected per premise.

Adult mosquitoes were collected with a sweep net, and/or mouth aspirator. All samples were labeled and brought to the Laboratory for Medical Entomology (LME), Faculty of Agriculture Novi Sad for identification. Mosquitoes were identified morphologically by using the diagnostic keys (Becker et al., 2003; Schaffner, 2003; Becker et al. 2010).

Latter on (2009 – 2011) ovitraps were distributed at used tire storage places, border crossings to Croatia, Montenegro and FYRO Macedonia, as well as on main resting places

on roads connecting Serbia with neighboring countries infested by Asian tiger mosquito and main flower importation companies in Novi Sad (usually 10 ovitraps per sampling site). The trap stations were kept fixed for the whole season and inspected every 7<sup>th</sup> to 14<sup>th</sup> day. Within each sampling site, the ovitrap was placed in a position of most probable occurrence of invasive species often not in accordance with standard requirements for trap placement (green, shaded, and easily accessible area). It was positioned on the ground, with a free space of at least 1 m above it. Each ovitrap consisted of a black plastic pot (capacity 400 ml; upper diameter: 8 cm), filled to about 2/3 of its height with about 285 ml of dechlorinated water. One 12.5 x 2.5 cm strip of masonite as egg deposition substrate. The weekly/biweekly check of the ovitraps provided for the replacement of the deposition substrate and of the dechlorinated water, after a careful cleaning in order to remove any eggs. Masonite strips were then delivered to the LME for classification, counting, hatching and breeding the mosquitoes to the 4<sup>th</sup> instar larvae or adult stage and identification (Becker et al., 2003; Schaffner, 2003; Becker et al. 2010).

## RESULTS

First specimen of invasive *St. albopicta* in former Federal Republic of Yugoslavia (FRY), now Montenegro, was registered in Podgorica on August 21<sup>st</sup> 2001. One last instar larva was found in water collected from used tires produced in France and imported from Germany. It was developed to a male adult in laboratory. During 2002, 168 larvae and 55 adults of *St. albopicta* were detected in 3 of 5 monitored spots in Podgorica including used tire shops and residential area downtown. While in the USA and EU the used tires are used as a fuel for power production, road surfacing, and recycling for carpet industry, in Montenegro they have been sold to be used in traffic again or some of them are used as a kind of a barrier for coastal protection.

In the year 2003 and 2004, *St. albopicta* spread to east and mid coastal part of Montenegro and around capital city of Podgorica. Next year, Asian tiger mosquito was detected close to Andrijevica at altitude of 850m that was first record on such high elevation in Europe, indicating possible adaptation to climatic conditions outside the limits of average temperature values foreseen for Europe at that time. The coldest month average temperature in Andrijevica is -2.6°C (January). Between 2006 and 2011, inspections were done usually in August and September, when mosquitoes are most abundant, mostly once per season and limited to high risk premises because of no budget available. Patchy distribution indicating spreading to Boka Kotorska bay and Luštica peninsula as well as places around Skadar Lake was recorded. From 2012 new project "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" was launched, allowing systematic approach aimed to depict both distribution and numerosity of *St. albopicta* across Montenegro in next three years. First year surveillance is focused to Montenegro coast. Until now Asian tiger mosquito is detected on 17 out of 40 sampling sites. Number of eggs per positive ovitrap in May 2012 ranged from 1 to 127, average 4.39.

In Serbia first egg detection was made on 1<sup>st</sup> September 2009 in ovitrap positioned at passport/custom control terminal of Batrovci border pass between Croatia and Serbia. The masonite strip from positive trap was brought to laboratory for eggs to hatch and after rearing of larvae and pupae, 22 adults of *St. albopicta* eclosed. That was the first detection of Asian tiger mosquito at country level. Surveillance was continued on weekly basis until 14<sup>th</sup> October 2009 but no new detections were made. Water containers in vicinity of border pass were also checked for presence of immature stages as well as human bait samplings

but no *St. albopicta* specimens had been detected. It seems that we were lucky enough to stop establishment by having the eggs deposited in one of our traps. Apart from border, sets of three to 10 ovitraps were positioned at used tire storage dump in La Farge cement factory, Beočin; three petrol/rest stop station at the highway Zagreb – Belgrade proximal to border pass; one restaurant visited by travellers from Montenegro coast and two flower importation companies in Novi Sad.

Same sampling setup was repeated next year, in weekly intervals from 13<sup>th</sup> of August until 20<sup>th</sup> of September but no new detections were made. Until 13<sup>th</sup> of September traps were positive for egg rafts, larvae and pupae of *Cx.pipiens pipiens* and larvae of hover flies (Diptera: Syrphidae).

In 2011, biweekly/weekly samplings were performed on border passes to Croatia (Batrovci), Montenegro (Jabuka and Gostun) and FYRO Macedonia (Preševo), using 10 ovitraps per sampling site. For inspection of all border passes two days of work and more than 1400km of travel were needed. Surveillance was done in five round trips, on 27-28 July, 4-5 August, 15-16 August, 24-25 August, 31 August- 1 September and 12-13 September 2011. All sampling sites except Batrovci were negative for the eggs of *St. albopicta*, so last four samplings (18<sup>th</sup> and 29<sup>th</sup> September and 4<sup>th</sup> and 11<sup>th</sup> October) were concentrated to this border pass only.

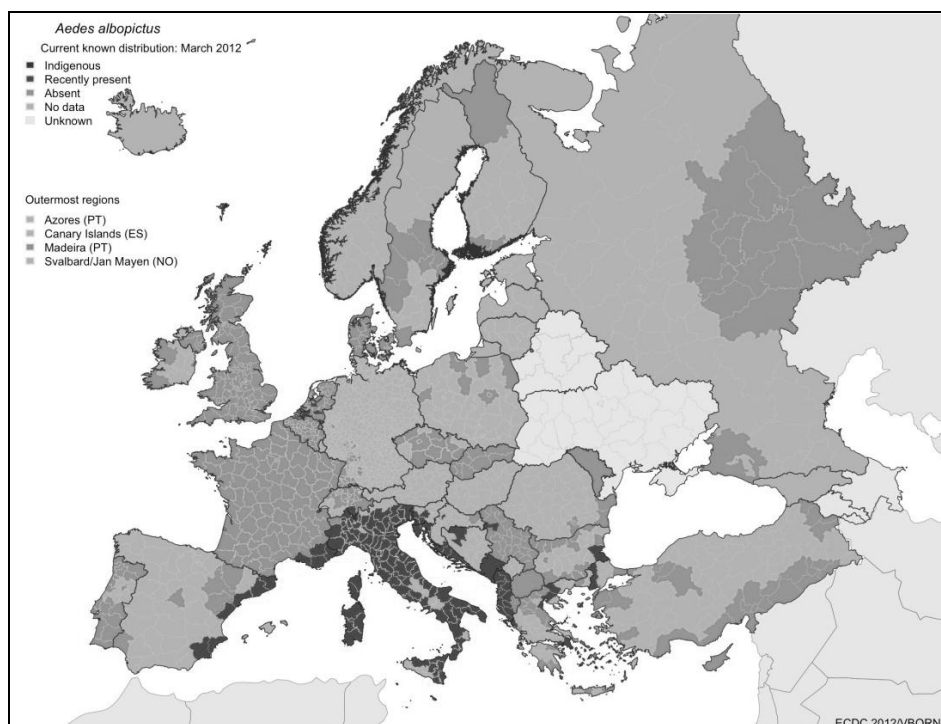
First eggs of *St. albopicta* at Batrovci were detected on 31<sup>st</sup> of August (32 eggs) and then on 18<sup>th</sup>(46 eggs) and 29<sup>th</sup> (5 eggs) September. On 4<sup>th</sup> October one *St. albopicta* female was sampled close to the ovitrap situated on concrete floor near the entrance to custom offices. No invasive species were detected on other border passes but egg rafts, larvae and pupae of *Cx.pipiens pipiens* were frequently found.

## DISCUSSION

After our findings of *St. albopicta* in a small used tire trade company in Podgorica in 2001 (Petrić et al., 2001), and also because no capacity to process all imported used tires in the safe way were available in Serbia, national government imposed amendment on Nature protection law that ban the importation of used tires in Serbia in 2007. It is likely that this legal decision has been protecting Serbia from introduction of Asian tiger mosquito for couple of years and will lower the risk of new introductions in the years to come.

The “Asian tiger mosquito”, *St. albopicta*, originating from Southeast Asia, has undergone a noteworthy expansion of its range in the last few decades (Hawley, 1988). Due to its immense invasive capacity, it is listed in the inventory of “100 of the World's Worst Invasive Alien Species” (<http://www.issg.org>). With the increase in the international trade of used tires, this species has spread across very large distances and between continents (Reiter, 1998). In Europe, it was first reported in Albania in 1979 (Adhami, and Murati, 1987) probably due to the country intense trade with China but did not spread around, most likely because of the trade isolation of the country at neighbouring countries level. Subsequent introduction was reported in Italy in 1990, where it was probably introduced through the import of used tires from the USA (Sabatini et al., 1990; Dalla Pozza and Majori, 1992). Over the next few years, the species rapidly dispersed to other regions of Italy (Romi, 1994), and it has now been reported in France - 1999 (Schaffner and Karch, 2000), Belgium - 2000 (Schaffner et al., 2004), Federal Republic of Yugoslavia, now Montenegro - 2001 (Petrić et al., 2001; Becker et al., 2003), Greece - 2003 (Samanidou-Voyadjoglou et al., 2005), Switzerland - 2003 (Flacio et al., 2004), Croatia - 2004 (Klobučar et al. 2006), Spain - 2004 (Aranda et al., 2006), Slovenia, Bosnia and

Herzegovina - 2005 (Scholte and Schaffner, 2007), the Netherlands - 2005 (Scholte et al., 2007), Germany - 2007 (Pluskota et al., 2008) Serbia - 2009 (Petrić, 2009), Bulgaria and Turkey – 2011 (Mikov, personal communication) (Fig.2).



**Figure 2.** Distribution of *St. albopicta* in Europe (VBORNET vector maps: <http://ecdc.europa.eu>).

The “Asian rock pool” or “Asian bush” mosquito, *Hl. japonica*, is an Asian species native to Japan, Korea, South China, Taiwan and the Russian Federation. In Europe, this species was established in Belgium and has successively been detected in Switzerland and Germany, where it is rapidly spreading (Schaffner et al., 2009; Becker et al., 2011).

The “African tiger mosquito” or “Yellow fever mosquito”, *St. aegypti*, has spread across almost all tropical and subtropical countries over the past four centuries. *St. aegypti* disappeared from Southern Europe at the beginning of the last century but recently, in 2004, was introduced in Madeira and has since started to spread around the Black Sea. It has also been introduced to the Netherlands through the used tire trade (Scholte et al., 2010).

The primary dispersal mode of these three invasive mosquito species by human activity has been through the transport of desiccation-resistant eggs in cargo. The most important types of goods responsible for this passive transport are used tires, which are generally stored outdoors and thus collect and store rain water that is indispensable for mosquito development (Knudsen, 1995). Businesses that process and/or trade used tires should be given a high priority for the monitoring of exotic fauna and flora. Another documented source of introduction is through ornamental plants, e.g., “Lucky Bamboo” (*Dracaena* spp.) from Southeast Asia, which is transported in containers with standing



water, making it an ideal insectaria in transit. “Lucky Bamboo” was the primary reason for the introduction of *St. albopicta* from Southeast Asia to California (Madon et al. 2004). Similarly, multiple introductions of the Asian tiger mosquito to the Netherlands in commercial horticultural greenhouses have been linked to the intensive trade of this plant (Scholte et al., 2007; Scholte et al., 2010).

Therefore, harbors, ports and inland air or road terminals that receive transoceanic containers from infested countries should be routinely monitored. Rest areas and parking lots along highways originating in areas infested with exotic species can also serve as sites of introduction (Flacio et al., 2004; Pluskota et al., 2008).

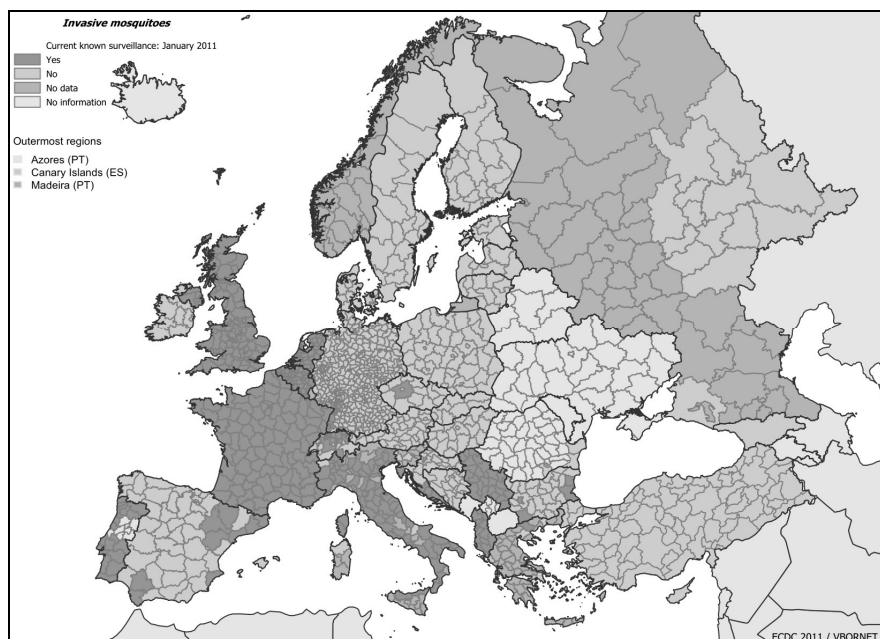
*St. aegypti* and *St. albopicta* are the primary and secondary vectors, respectively, for Dengue fever (DF) and Dengue hemorrhagic fever (DHF), which affect more than 40 % of the human population worldwide, especially in mega-cities of the tropics (Halstead, 1980, 1982, 1992; Becker et al., 1991; Gratz, 1999). *St. albopicta* is the most important vector for the Chikungunya virus (Reiter et al., 2006). Recently, this species was involved in the transmission of Chikungunya virus to humans in Italy in 2007 and was also most likely involved in the first confirmed autochthonous dengue cases in France and Croatia in 2010 (Beltrame et al., 2007; Becker et al., 2010; Schmidt-Chanasit, 2010; Gjenero-Margan et al., 2011). In addition to Dengue and Chikungunya, other viruses such as Batai, Inkoo, Lednice, Sindbis, Tahyna, Usutu and West Nile have shown some activity, and the Rift Valley and Japanese encephalitis viruses are likewise threatening human health in Europe (Becker et al., 2010).

The capacity of Serbia to detect the early presence of invasive species and define their abundance and colonized area needs to be rapidly improved in order to increase the chances of early detection and elimination of invaders at the beginning of the colonization process and/or to develop efficient control programs. Moreover, in areas where the invading species is established, monitoring of further spread and abundance is needed for timely risk assessment of arbovirus transmission.

It has been demonstrated on several occasions within different countries and environmental conditions that it is possible, and perhaps highly convenient in term of cost-benefit balance, to eliminate an invading mosquito species by promptly applying intensive suppression methods if the colonized area is still well delimited.

At least some kinds of surveillance and monitoring networks (research and/or control based) are already organized in many European countries, including Albania, Belgium, Bulgaria, Croatia, the Czech Republic, France, Germany, Greece, Italy, Montenegro (through a research project, started in 2012), the Netherlands, Portugal, Serbia (through the research projects, started in 2009), Slovenia, Spain, Switzerland and the United Kingdom (Fig. 3).

European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden has been supporting several projects that aim to increase the capacities of European countries for surveillance and control of invasive mosquito species and vector-borne diseases: (i) the TigerMaps project, which included a multi-model approach to model and predict the spread of *St. albopicta* in Europe taking into consideration the current presence/absence data, expert knowledge and a variety of IPCC-derived climate scenarios; (ii) VBORNET, the European Network for Arthropod Vector Surveillance for Human Public Health; (iii) Vi-Map, which aims to map European health vulnerabilities to climate change related to communicable disease and (iv) MosqInvade project which outcomes are Guidelines for the surveillance of invasive mosquitoes in Europe (Schaffner et al., 2012).



**Figure 3.** Current known surveillance activities in Europe  
(VBORNET: <http://ecdc.europa.eu>)

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